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Colorectal Cancer: Molecular Genetic Studies and Their Future Clinical Applications

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INTRODUCTION

A genetic basis for the development of cancer has long been recognized, but it is only over the past two decades that direct evidence has been obtained to support the proposal that cancer is a genetic disease. Indeed, present data suggest that cancers arise through a multi-step evolution driven by somatic mutation of cellular genes and clonal selection of variant progeny with increasingly more aggressive growth properties. While molecular genetic studies have provided insights into the pathogenesis of many different tumor types, our understanding of cancer at a genetic level is probably most advanced in colorectal cancer.

Approximately 160,000 cases of colon and rectal cancer were diagnosed in 1994, and more than 45% of these patients will die from their disease. These statistics reflect the fact that, as for most common epithelial tumor types, limited progress has been made in the treatment of advanced colorectal cancer with chemotherapeutic agents. In addition, relatively little is known about specific dietary or pharmacologic strategies that will prevent or at least delay the development of colorectal cancer. Nevertheless, despite the present difficulties in the prevention and treatment of colorectal cancer, colorectal tumors have proven to be a rich experimental system for study of the nature, role, and origins of mutations in a common human cancer because of their natural history and the inherited syndromes predisposing to cancer development. The insights gained from these studies have not only shed light on the pathogenesis of colorectal and other cancer types, but should provide the foundation for important advances in our ability to prevent, detect, and treat the disease.

Enormous progress has been made in the identification and characterization of the genetic alterations present in cancer in general and colorectal cancer in particular, so that it will not be possible to review all of these data. Rather, the primary aims of this review will be the following: i) to summarize the general properties of the genes that are mutated in colorectal cancer, including oncogenes, tumor suppressor genes, and DNA damage recognition and repair genes; ii) to describe some of the genetic defects present in the germline of those with inherited predisposition to colorectal cancer; iii) to review some of the somatic mutations that are frequently seen in colorectal tumors; and iv) to outline the means by which the information gleaned from the molecular genetic studies may be applied to improve the prevention, detection, and treatment of colorectal cancer.

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ONCOGENES, TUMOR SUPPRESSOR GENES, AND DNA DAMAGE RECOGNITION AND REPAIR GENES

When mutated, oncogenes act in a positive fashion to promote tumor development [1,2]. Their normal-functioning cellular counterparts are often termed proto-oncogenes, but this term should not be interpreted as implying that these genes lie dormant in the cell with the sole purpose of promoting tumor formation. Rather, the term refers to the fact that in cancer cells, specific mutations alter the normal structure and/or expression pattern of proto-oncogenes, generating oncogenic variant forms with abnormal function [1,2]. In genetic terms, the oncogenic alleles have acquired gain-of-function mutations compared to the corresponding normal proto-oncogene alleles. The proteins encoded by the various proto-oncogenes are important regulators of many different growth regulatory pathways and are found in virtually all subcellular compartments, including the nucleus, cytoplasm, and cell surface [1–3]. Finally, it is important to note that while more than 50 proto-oncogenes have been identified through experimental laboratory studies, less than 20 of these genes are frequently mutated in common human cancers [4].

Activating mutations generate oncogenic alleles from proto-oncogenes, while tumor suppressor genes contribute to tumor development as a result of mutations inactivating their normal functions [4–6]. The term antionco-

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genes is sometimes used to refer to the tumor suppressor class of genes. However, the term suggests that these genes act in direct opposition to oncogenes. A subset of the tumor suppressor genes may function in such a fashion, but this is not necessarily a general principle. Therefore, tumor suppressor genes will be defined as the class of genes in which loss-of-function mutations in both alleles of the gene contribute directly to the tumorigenic process in vivo. At present, fewer than 10 tumor suppressor and candidate tumor suppressor genes have been identified, although this class of genes may be nearly as sizable as the oncogenes [4,6].

Over the past two years, mutations in a third class of genes—the DNA damage recognition and repair genes—have been established to have a critical role in tumorigenesis [7–12]. Like the tumor suppressor genes, these genes are affected by loss-of-function mutations during tumor development. Recognition and repair genes have a more passive role in the processes controlling growth, unlike the tumor suppressor genes. The latter may actively function to regulate cell growth in response to inhibitory or differentiation signals, or may arrest cell growth or cause programmed cell death (apoptosis), following DNA damage. Specifically, loss-of-function mutations in recognition and repair genes appear to promote the acquisition of mutations in other cellular genes (including perhaps oncogenes and tumor suppressor genes) in affected cells. Germline mutations in a subset of these genes are responsible for the inherited predisposition and other tumors in patients with hereditary nonpolyposis colorectal cancer syndromes [8–12], and some of these findings will be discussed below.

THE GENETICS OF HEREDITARY COLORECTAL CANCER

Two distinct inherited syndromes predisposing to colorectal cancer—familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC)—have provided critical insights into mutations underlying tumorigenesis. FAP, an autosomal dominant syndrome, is estimated to affect about one in 10,000 in the U.S. and may account for about 0.5% of all colorectal cancer cases [13]. Those individuals inheriting a mutant adenomatous polyposis coli (*APC*) allele have very high likelihood of developing 100 or more adenomatous colorectal polyps by their third to fourth decades of life, and thus a very high risk of developing one or more colorectal cancers. The location of the *APC* gene on chromosome 5q was first suggested in 1986 by cytogenetic studies demonstrating an interstitial deletion on 5q in a patient with polyposis and mental retardation. This location was subsequently confirmed in 1987 by linkage analyses carried out on many families with polyposis [14–16]. Since the identification of the *APC* gene in 1991, germline mutations have been identified in one of the two *APC* alleles from affected individuals of about 80% of the polyposis kindreds studied [17–21]. The vast majority of the mutations observed are nonsense mutations or insertion or deletion mutations that lead to a truncated, and hence inactivated, APC protein product. Several allelic variants of familial polyposis have been described, including Gardner syndrome and attenuated APC (AAPC) in which affected individuals may develop as few as a dozen adenomatous polyps by age 50. Preliminary findings suggest that the mutations in those with AAPC are located in more 5′ regions of the *APC* gene than in those individuals with more classical features of polyposis [22]. In contrast to apparent genotype-phenotype correlation for those with the AAPC syndrome, the basis for the predisposition to extracolonic tumors in those with the variant Gardner syndrome has not yet been elucidated.

Although germline mutation of one *APC* allele has only been seen in those with FAP or variant syndromes, somatic mutations in the *APC* gene have been identified in more than two-thirds of adenoma and carcinoma specimens of the sporadic type [23,24]. Furthermore, somatic APC mutations are as prevalent in very small (early) adenomas as they are in carcinomas, and on this basis it has been suggested that APC mutation may be the initiating event in the majority of colorectal tumors with malignant potential. Similar to the inherited mutations identified, the vast majority of the somatic mutations in the *APC* gene appear to result in the synthesis of prematurely truncated APC protein products. Furthermore, while some mutant *APC* alleles may encode defective proteins that inhibit the function of the wild-type APC protein or other cellular proteins, inactivation of both *APC* alleles has been demonstrated in a sizeable fraction of colorectal tumors arising in those with polyposis, as well as in sporadic cases [25].

The *APC* gene encodes a very large protein with a relative molecular mass greater than 300,000 and this protein does not bear particularly strong sequence similarity to well-characterized proteins [17,20]. Recent studies suggest that APC is localized in the cytoplasm and complexed with other cellular proteins, including α and β-catenin, two adherens junction proteins [26,27]. Given the critical role of the adherens junction in epithelial cell-cell interactions and perhaps even epithelial tissue homeostasis (e.g., maintenance of cell polarity), defects in adherens junction function may be among the critical cellular defects resulting from loss-of-function mutations in the *APC* gene in colorectal tumors.

While polyposis is a rare cause of colorectal cancer, the HNPCC syndromes may account for 5–10% of all colorectal cancer cases [28]. The clinical features of the HNPCC syndrome are somewhat heterogeneous. Cancers of other organs, besides the colon and rectum, are also seen in some affected by this syndrome [29]. It also is

now apparent that significant genetic heterogeneity exists for the inherited predisposition to tumors in those with the HNPCC syndrome. Germline mutations in the human homologs of four different DNA mismatch repair genes (*MSH2, MLH1, PMS1,* and *PMS2*) have been identified in affected individuals from different HNPCC kindreds [8–12,30]. It appears that mutations in the *MSH2* gene may be the basis for cancer predisposition in about one third of the families with characteristic clinical features of the HNPCC syndrome [12].

Study of colorectal tumors arising in those with HNPCC has revealed that somatic mutations inactivate the remaining wild-type allele of the particular mismatch repair gene that is mutated in the patient's germline. Furthermore, the tumors appear to display a mutator or replication error (so-called "RER+") phenotype, with frequent alterations of dinucleotide repeat sequences (e.g., CA or TA repeats) [31]. Inactivation of both alleles of a critical damage DNA recognition and repair gene has been shown to lead to a decreased ability to repair DNA mismatches that arise in a dividing cell. The net result might be a "mutator" phenotype in some initiated/preneoplastic cells. Moreover, consistent with the clinical observations that those with HNPCC develop relatively few adenomatous polyps but are at high risk for colorectal cancer, inactivation of both alleles of a DNA mismatch repair gene may promote the more rapid expansion of an initiated clone of preneoplastic cells by causing multiple transition and transversion mutations, as well as microsatellite alterations. Some of these mutations may activate oncogenes or inactivate tumor suppressor genes. Finally, although somatic mutations in mismatch repair genes have been observed predominantly in tumors from patients who are known to harbor a germline mutation in one allele, it is interesting to note that about 15–20% of apparently sporadic colorectal cancers, also display the RER+ phenotype [32]. Thus, defects in DNA damage and mismatch repair genes may have a critical role in a sizable fraction of colorectal tumors.

THE ROLE OF SOMATIC MUTATIONS IN ONCOGENES AND TUMOR SUPPRESSOR GENES IN TUMOR PROGRESSION

In addition to inherited and somatic mutations in the *APC* and the DNA damage recognition and repair genes, somatic mutations in other genes appear to be critical in tumorigenesis. The proto-oncogene most frequently altered in colorectal tumors is K-*RAS,* which is activated by point mutation at codon 12 or 13 in nearly 50% of carcinomas and 50% of adenomas greater than 1 cm in size [33]. Other studies suggest that K-*RAS* mutations are detected frequently in adenomas with increased dysplasia [34]. Thus, K-*RAS* mutations are associated with two histopathologic features—increased size and dysplasia—which are thought to be predictive of progression of the adenomatous polyp to cancer. Further studies will be necessary to address the precise role and temporal occurrence of *RAS* mutations in colorectal tumorigenesis, but two additional points are worth noting. K-*RAS* mutations appear to be prevalent in colonic lesions (i.e., aberrant crypt foci) that may have very reduced or no malignant potential [35–37]. Nevertheless, loss of mutant K-*RAS* activity in advanced colorectal cancer cells appears to abrogate their tumorigenicity in in vitro studies [38]. In contrast to K-*RAS* mutations, somatic mutations in other proto-oncogenes, such as N-*RAS, MYC, MYB,* and *HER-2/neu,* have been found in only a small percentage of colorectal cancers [39].

Tumor suppressor genes may be inactivated by several mechanisms, including localized mutations or deletions of large chromosomal regions. Typically such deletions involve one of the two parental chromosome sets, or alleles, present in normal cells and are thus referred to as losses of heterozygosity (LOH) or allelic losses. In accord with a hypothesis originally proposed by Knudson, LOH usually unmasks a more subtle recessive mutation in the retained allele of the tumor suppressor gene [4–6, 40]. By characterizing the chromosomal regions affected by LOH in colorectal cancers, alterations in several tumor suppressor genes have been identified [39,41]. For example, allelic losses affecting chromosome 17p can be detected in greater than 75% of carcinomas, although 17p LOH is infrequent in adenomas [33]. Wild-type *p53* alleles are presumed to be targeted for inactivation by these allelic losses, as the remaining *p53* allele is very frequently mutated in cases with 17p LOH, often in codons 175, 248, or 273 [42,43]. Only a very small subset of colorectal tumors that have not suffered 17p LOH have a mutant *p53* allele. Both mutation of one *p53* allele and loss of the remaining wild-type allele occur frequently only in later stage colorectal tumors—perhaps during the transition from adenoma to carcinoma. Presently, it appears that the loss of p53 function as a transcriptional activator may affect the regulation of genes that inhibit cell cycle progression, either those involved constitutively in cell cycle or in response to DNA damage [44–48]. These genes may include *WAF1/CIP1/p21* and *GADD45* [45–47]. The loss of p53 function may also affect pathways regulating apoptosis in the tumor cells [49]. In addition to a loss of wild-type function, some p53 mutant proteins may have a "gain-of-function" that contributes to the phenotype of advanced cancer cells [44].

Chromosome 18q LOH can be detected in more than 70% of colorectal primary cancers, in about 50% of advanced adenomas, and infrequently in earlier stage adenomas [33]. The prevalence of 18q LOH rises to nearly 100% in hepatic metastases of colorectal primaries, suggesting a role in tumor progression and metastasis [50]. In addition, patients whose primary colorectal cancers

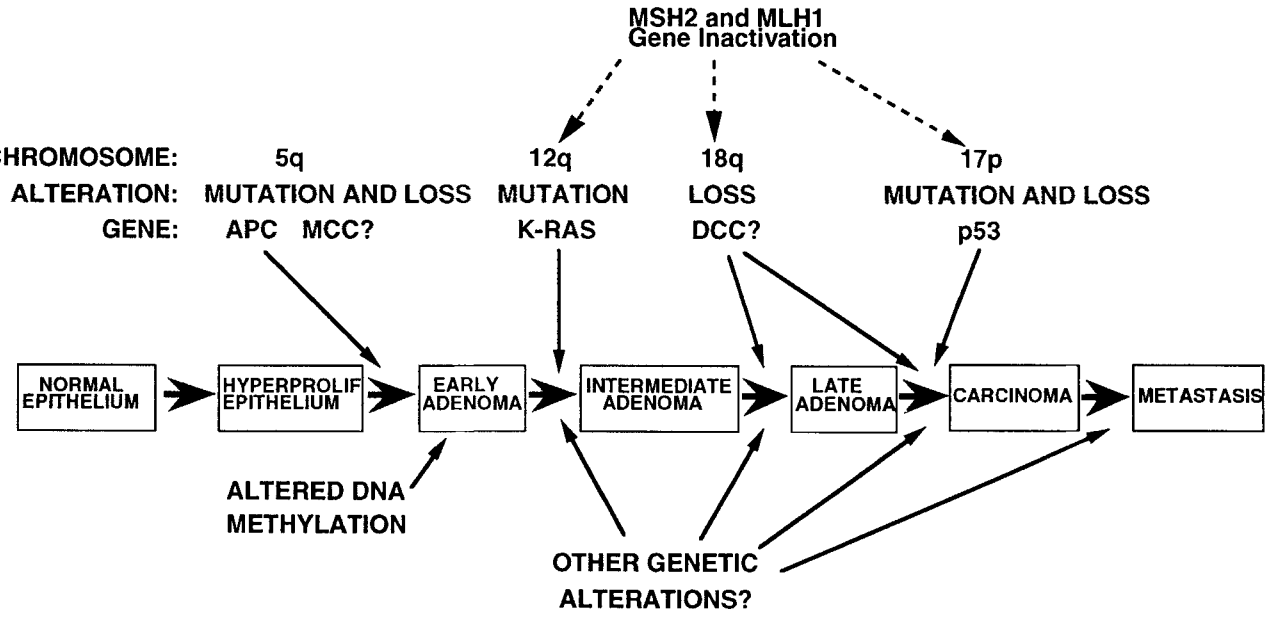

Fig. 1. A genetic model of colorectal cancer. A large body of clinical and histopathological evidence supports the notion that the majority of colorectal cancers arise from adenomatous polyps over a period of years or even decades. Shown in the figure are three stages of adenoma, distinguished from one another by histopathological features. The inherited and somatic genetic alterations found at various stages of colorectal tumorigenesis are indicated (with the specific gene affected, its chromosomal location, and the nature of the alteration in the gene) and are discussed in more detail in the text. It is thought that inactivation of DNA damage recognition and repair genes (e.g., *MSH2* and *MLH*) in those with HNPCC may lead to the more rapid acquisition of mutations in oncogenes such as K-*RAS* or tumor suppressor genes such as *p53* and *DCC*. Figure modified from Fearon and Vogelstein: A genetic model for colorectal tumorigenesis. Cell 61:759–767, 1990.

have 18q LOH have an increased likelihood of distant metastasis and death from their disease [51]. Several lines of evidence suggest that a candidate tumor suppressor gene on 18q termed *DCC* (for *d*eleted in *c*olorectal *c*ancer) may be a gene targeted for inactivation by the allelic losses [52–55]. *DCC* is contained within the common region of LOH on 18q, its expression is markedly decreased or absent in the majority of colorectal cancers and cell lines, and somatic mutations in the gene have been identified in a subset of cases. However, localized somatic mutations in the remaining *DCC* allele have not yet been identified in the majority of cases with 18q LOH. The difficulty in identifying localized mutations in *DCC* may be due, in part, to the fact that *DCC* spans greater than 1.35 million base pairs at chromosome band 18q21.1. The identification of mutations in such a large gene poses both theoretical and practical problems. *DCC* encodes a 1447 amino acid transmembrane protein whose 1100 amino acid extracellular domain bears similarity to the neural cell adhesion molecule (NCAM) family of proteins [54,55]. Based on its similarity to the N-CAMs, DCC may function to regulate cell growth or differentiation through cell-cell or cell-extracellular matrix interactions. Loss of function may account for some of the invasive and metastatic properties of advanced colorectal cancer cells.

Undoubtedly, besides those mutations described above, additional mutations in other cellular genes remain to be identified and characterized in colorectal tumors. Moreover, some genes with critical roles in the tumor process may not be affected by somatic mutations. Rather, their expression may be altered as a result of mutations in other genes, such as K-*RAS, APC,* or *p53.* Finally, although it is likely to be overly simplistic, a genetic model for colorectal cancer that relates the genetic alterations to the natural history of the disease has been proposed (Fig. 1).

CLINICAL APPLICATIONS OF THE MOLECULAR GENETIC STUDIES

Only a few of the potential clinical applications of the molecular genetic studies will be considered here, but the applications can be roughly grouped into: presymptomatic diagnosis, early detection, improved prognostication and patient stratification, and treatment.

The identification and characterization of inherited genetic alterations that predispose to the development of colorectal tumors should prove useful for genetic counseling of individuals from families with inherited colorectal cancer. For example, individuals from polyposis kindreds who are found not to have inherited a mutant *APC* allele will be spared anxiety and frequent clinical examinations in their adolescent and early adult years. Those who have inherited the disease gene can be closely monitored, and

offered a definitive colonic resection at an appropriate time. In the future, they might be treated with chemopreventive regimes. Similarly, those from HNPCC kindreds who harbor germline mutations in a DNA mismatch repair gene can also be more effectively counseled and monitored. However, it should be noted that presymptomatic testing for cancer in general and, specifically, population screening for germline mutations predisposing to colorectal cancer, is presently complicated by many ethical, legal, and practical issues.

In addition to identifying those at markedly increased risk of colorectal cancer as a result of inherited mutations, it may someday be possible to apply molecular genetic studies to the early detection of colorectal tumors in the general population. In this regard, studies of DNA isolated from stool samples of patients suggest that mutated K-*RAS* alleles can be detected in cells shed from large adenomas and carcinomas [56]. Other possible clinical applications of the molecular genetic studies include improved/increased prognostic information about the likelihood of tumor recurrence and subsequent distant metastasis, and perhaps improved stratification of patients for adjuvant chemotherapeutic intervention. For example, it has already been shown that those patients whose colorectal cancers have 18q LOH and who did not have cancer detected in regional lymph nodes at the time of diagnosis (i.e., Stage II) have a markedly decreased 5-year survival compared to patients with tumors of similar stage and no 18q LOH [51]. Finally, an implied promise of the present molecular genetic studies to elucidate the pathogenesis of cancer is that such studies will identify novel targets for new chemotherapeutic agents. In this regard, recent studies suggest that small-molecule inhibitors of RAS function may have some efficacy against colorectal cells harboring K-*RAS* gene mutations [57]. Further studies may identify other novel agents directed at one or more of the altered oncogene and tumor suppressor gene products in colorectal cancers. An optimistic view is that future molecular genetic studies of colorectal tumorigenesis will not only increase our understanding of colorectal cancer pathogenesis, but will lead to improvements in the care and management of patients with cancer.

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